

Effects of Dehydration during Cycling on Skeletal Muscle Metabolism in Females

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ABSTRACT

LOGAN-SPRENGER, H. M., G. J. F. HEIGENHAUSER, K. J. KILLIAN, and L. L. SPRIET. Effects of Dehydration during Cycling on Skeletal Muscle Metabolism in Females. *Med. Sci. Sports Exerc.*, Vol. 44, No. 10, pp. 1949–1957, 2012. **Introduction:** This study investigated the effects of progressive dehydration on the time course of changes to whole body substrate oxidation and skeletal muscle metabolism during 120 min of cycling in hydrated females. **Methods:** Subjects ($n = 9$) cycled for 120 min at approximately 65% $\dot{V}O_{2\text{peak}}$ on two occasions: with no fluid (DEH) and with fluid (HYD) replacement to match sweat losses. Venous blood samples were taken at rest and every 20 min and muscle biopsies taken at 0, 60, and 120 min of exercise. **Results:** DEH subjects lost 0.9% body mass from 0 to 60 min and 1.1% from 60 to 120 min (2.0% total). HR and core temperature (T_c) were significantly greater from 30 to 120 min, plasma volume (Pvol) loss from 40 to 120 min, and RPE from 60 to 120 min in the DEH trial. There were no differences in $\dot{V}O_2$ or sweat loss between trials. RER (HYD, 0.85 ± 0.01 , vs. DEH, 0.87 ± 0.01) and total CHO oxidation (175 ± 17 vs. 191 ± 17 g) were higher in the DEH trial. Blood (La) was significantly higher in the DEH trial, with no change in plasma free fatty acid and epinephrine concentrations. Muscle glycogenolysis was 31% greater in the DEH trial (252 ± 49 vs. 330 ± 33 mmol·kg⁻¹ dry muscle), and muscle (La) was also higher at 60 min. **Conclusion:** Progressive dehydration significantly increased HR, T_c , RPE, Pvol loss, whole body CHO oxidation, and muscle glycogenolysis, and these changes were already apparent in the first hour of exercise when body mass losses were $\leq 1\%$. The increased muscle glycogenolysis with DEH appeared to be due to increased core and muscle temperature, secondary to less efficient movement of heat from the core to the periphery. **Key Words:** HYDRATION, EXERCISE, FLUID INTAKE, BODY MASS LOSS, SWEAT RATE, SUBSTRATE OXIDATION

Mild dehydration during exercise can be a major concern for athletes because it may lead to premature fatigue. The cardiovascular and thermoregulatory consequences of exercising dehydrated are well documented with the magnitude of these effects directly proportional to the degree of dehydration (2,14,21,23,25,27,28,30,34,36,38,39). As little as a 2% loss in body mass (BM) due to dehydration has been consistently reported to result in elevated HR, core temperature (T_c), rate of perceived exertion, and plasma osmolality. However, little research has investigated the effects of exercise-induced dehydration on whole body substrate use and skeletal muscle metabolism. Hargreaves et al. (17) investigated the effects of exercise-induced dehydration on muscle metabolism in males in a

temperate environment (20°C–22°C) and reported that a 3% BM loss resulted in a significantly higher rectal and muscle temperature (T_m) at 120 min of exercise, with no difference in rectal temperature between trials at any other time point during exercise. The respiratory exchange ratio was also significantly higher in the fluid-restricted trial after 60 and 120 min of exercise, with the difference between trials being greater in the second hour of cycling. The study reported a 16% greater glycogen use during the 120 min of exercise (17). Other studies reported similar findings but have been performed in the heat. For example, Gonzalez-Alonso et al. (15) had seven males cycle until volitional exhaustion (135 ± 4 min) in the heat (35°C) while developing progressive dehydration to approximately 3.9% BM loss. They reported increased carbohydrate oxidation, muscle glycogen use (45% greater), muscle lactate accumulation, and net lactate release across the contracting leg compared with being euhydrated. More recently, Febbraio (9) reviewed the relevant literature examining exercise in the heat and concluded that when T_m is higher than control during exercise, there is augmented muscle glycogenolysis. However, some studies did not control for hydration status and were all conducted in males. Presently, there are no studies investigating the time course of progressive exercise-induced dehydration on whole body substrate oxidation and skeletal muscle metabolism in females.

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It has been suggested that women thermoregulate less effectively because of higher T_{c} during the same load exercise compared with males (26,40). Work by Gagnon et al. (13) also suggests that females experience a quicker rise in T_{c} during exercise, which may accelerate muscle glycogen use. The effect of the heightened T_{c} during prolonged exercise in females coupled with the thermal stress associated with progressive dehydration on substrate oxidation and muscle metabolism has yet to be elucidated.

In light of the small amount of information examining the effects of mild dehydration (1%–2%) on muscle metabolism in general, we investigated the effects of progressive exercise-induced dehydration in hydrated females to determine the time course of changes to physiological responses and skeletal muscle metabolism. We hypothesized that because female subjects progressively dehydrated during exercise and T_{c} increased above the increase in the hydrated state, there would be a greater reliance on whole body carbohydrate oxidation and muscle glycogenolysis during prolonged exercise. As well, we expected that these differences would be augmented in the second hour of exercise in the dehydrated trial.

METHODS

Subjects. Nine recreationally active females (mean age, 21.7 ± 0.6 yr; height, 155.7 ± 2.8 cm; weight, 58.8 ± 2.8 kg; and $\dot{V}O_{2\text{peak}}$, 2.9 ± 0.2 L·min⁻¹, participated in the study. All subjects engaged in recreational physical activity 2–3 d·wk⁻¹ and were taking oral contraceptives. Testing occurred at a time during their menstrual cycle other than ovulation. Subjects were informed both verbally and in writing of the experimental protocol and potential risks before giving their written consent to participate. The Research Ethics Boards of the University of Guelph and McMaster University approved the study.

STUDY DESIGN

Preexperimental protocol. In preparation for the experiment, subjects visited the laboratory on three separate occasions. On the first visit, subjects performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (LODE Excalibur; Quinton Instrument, Groningen, The Netherlands) for the determination of $\dot{V}O_{2\text{peak}}$. Respiratory gases were collected and analyzed using a metabolic cart (MOXUS metabolic system; AEI Technologies, Pittsburgh, PA). After a 30-min break, subjects cycled for approximately 20 min at approximately 65% $\dot{V}O_{2\text{peak}}$ to establish the power output for the subsequent 120-min trials.

On two subsequent occasions, subjects reported to the laboratory for practice trials and cycled at approximately 65% $\dot{V}O_{2\text{peak}}$ for 120 min without fluid (DEH) or with fluid (HYD) to replace sweat losses. DEH trials occurred first to ascertain sweat losses over the 120-min trial and determine how much fluid subjects needed to drink throughout the HYD

trial to maintain fluid balance. All subjects abstained from strenuous exercise and caffeine and recorded their diet in the 24 h before the trials. Two hours before the practice rides, subjects ingested a meal provided for them (790 kcal; 144 g of carbohydrate, 35 g of fat, and 19 g of protein) and 250 mL of fluid. Subjects also drank 300 mL of water 90 and 45 min before each trial to ensure they were well hydrated before cycling. Upon arrival to the laboratory, subjects voided their bladder and provided a small midstream urine sample to determine urine specific gravity (USG) and completely voided their bladder. A pretrial BM measurement was made wearing only dry shorts and a sports bra. After 60 and 120 min of exercise, subjects stopped cycling and dismounted the cycle ergometer, removed their shoes and shirt, towed dry, and were weighed wearing only shorts and a sports bra for the determination of sweat loss during the previous hour of exercise. At 60 min, subjects put on a dry T-shirt and recommenced cycling. Any urine produced was collected during each trial to account for total sweat loss using the following equation:

$$\begin{aligned} \text{sweat loss} = & (\text{pre-BM} - \text{post-BM (kg)}) \\ & + \text{fluid intake (mL)} \\ & - \text{urine loss (mL)} \end{aligned}$$

Three-minute respiratory gas measurements were collected every 20 min during exercise to determine the volume of oxygen consumed ($\dot{V}O_2$), to determine the volume of carbon dioxide produced ($\dot{V}CO_2$), and to calculate the respiratory exchange ratio (29) and whole body CHO and fat oxidation with the use of the nonprotein RER table and the following equations:

$$\text{CHO oxidation (g)} = 4.585 (\dot{V}CO_2) - 3.226 (\dot{V}O_2)$$

and

$$\text{fat oxidation (g)} = 1.695 (\dot{V}O_2) - 1.701 (\dot{V}CO_2) \quad (12,29).$$

Practice trials were separated by 5–7 d.

Experimental protocol. Subjects arrived to the laboratory on two occasions for the actual experiment. During the experimental trials, subjects cycled at approximately 65% $\dot{V}O_{2\text{peak}}$ for 120 min with fluid to match sweat losses (HYD) or without fluid (DEH). Subjects replicated the same procedure as described above for the practice trials. In addition, HR was collected using a Polar RS400 downloadable HR monitor (Polar Electro, Lachine, QC), and T_{c} was determined using an individually calibrated ingestible thermistor (HQ Inc., Palmetto, FL) that was ingested 3–5 h before each trial. Before exercise, a Teflon catheter was inserted into an antecubital vein for blood sampling and was flushed with 0.9% saline to maintain patency. One leg was also prepared for percutaneous needle biopsy sampling of the vastus lateralis muscle by the Bergström technique (4). Three incisions were made in the skin and deep fascia under local anesthesia (2% xylocaine without epinephrine (EPI)) for three separate biopsies. Immediately before exercise, a venous

blood (approximately 5 mL) sample and a muscle biopsy were obtained while the subject rested on a bed. All muscle samples were immediately frozen in the needle in liquid nitrogen and stored in liquid nitrogen for subsequent analyses. Subjects then cycled for 120 min at approximately 65% $\dot{V}O_{2peak}$ at a constant cadence (80–95 rpm). Venous blood samples were obtained at 20, 40, 60, 80, 100, and 120 min of exercise. HR, Tc, and RPE were recorded every 15 min during exercise. RPE was determined using the Borg scale (rating, 6–20) (5). During the HYD trial, subjects were given fluid every 15 min to match sweat loss and drank the fluid after HR, Tc, and RPE measurements were recorded. At 60 and 120 min, the subject stopped cycling and a muscle biopsy was taken with the subject sitting on the cycle ergometer. After the muscle biopsy was taken, subjects removed their shoes and shirt, towed dry, and were weighed for the determination of BM loss over the previous 60 min of exercise. The same procedure was replicated for the second trial, with muscle biopsies taken from the opposite leg, and the trials were randomized and separated by 7 d.

ANALYSES

Trial conditions. Laboratory temperature ($^{\circ}\text{C}$) and relative humidity (%) were measured using a digital thermometer (Fisher Scientific, Ottawa, ON). USG was measured via a hand-held pocket refractometer (Model PAL-10S; ATAGO USA Inc., Bellevue, WA) to assess hydration status from the preexercise urine sample. The refractometer was calibrated with distilled water before each measurement. Stover et al. (35) reported that USG measured with refractometry strongly correlated with urine osmolality ($r = 0.995$), and a USG of 1.020 correlated with urine osmolality of approximately 800 mOsm $\cdot\text{kg}^{-1}$. In light of this and the published position stand from the American College of Sports Medicine, a USG below 1.020 was considered to indicate a hydrated state (30).

Blood measurements. Venous blood was collected in sodium heparin tubes. A portion of whole blood (200 μL) was added to 1 mL of 0.6 M perchloric acid and centrifuged. The supernatant was stored at -20°C and later analyzed for blood glucose and lactate with fluorometric techniques (3). A second portion (1.5 mL) was centrifuged, and the supernatant was analyzed for plasma free fatty acids (FFAs) with an enzymatic colorimetric technique (NEFA C test kit; Wako Chemicals, Richmond, VA). A third portion (1.5 mL) was added to 30 mL of ethylene glycol tetraacetic acid and reduced glutathione and centrifuged (10,000g) for 3 min, and the supernatant was analyzed for EPI with an enzymatic

immunoassay kit (EPI RIA kit; Rocky Mountain Diagnostics Inc., Colorado Springs, CO). The remaining venous blood was used for the determination of whole blood hemoglobin (Hb) and hematocrit (Hct). Hb was measured in duplicate using an automated blood analysis machine (OSM3 Hemoximeter; Radiometer, Copenhagen, Denmark). Hct was measured in triplicate using capillary tubes and a micro-Hct centrifuge and reader (microcapillary reader; Damon/IEC Division, Needham Heights, MA). The percentage plasma volume change (%Pvol) was calculated using whole blood Hb and Hct measurements (7).

Muscle metabolites. Each muscle biopsy was freeze dried, powdered, and dissected free of visible connective tissue, fat, and blood. One aliquot of freeze-dried powdered muscle (approximately 10 mg) was extracted in 0.5 M HClO_4 –1 mM EDTA and neutralized with 2.2 M KHCO_3 . The supernatant was used to measure phosphocreatine (PCr), creatine (Cr), adenosine triphosphate (ATP), and lactate. Muscle metabolites were normalized to the highest total Cr content measured from all biopsies from each subject. Muscle glycogen content was determined in duplicate using two additional aliquots of freeze-dried muscle (2–4 mg). Glycogen was extracted in 0.1 M NaOH and neutralized with 0.1 M HCl–0.2 M citric acid–0.2 M Na_2PO_4 , and amyloglucosidase was added to degrade glycogen to glucose, which was measured spectrophotometrically and normalized for total Cr (3).

Muscle calculations. Free adenosine diphosphate (ADP_f) and free AMP (AMP_f) contents were calculated by assuming equilibrium of the Cr kinase and adenylate kinase reactions (8). Specifically, ADP_f was calculated using the measured ATP, Cr, and PCr values, an estimated H^+ concentration, and the Cr kinase constant of 1.66×10^9 (26). AMP_f was calculated from the estimated ADP_f and measured ATP content using the adenylate kinase equilibrium constant of 1.05.

STATISTICAL ANALYSIS

All data were tested for normality of distribution and presented as the mean $\pm$ SE. Time versus trial data were assessed using a two-way ANOVA, and specific differences were located using the Student–Newman–Keuls *post hoc* test. A paired *t*-test was used to compare single parameter data between trials. Statistical significance was accepted as $P < 0.05$.

RESULTS

Trial conditions. No significant pretrial differences existed between the HYD and DEH trials for laboratory

TABLE 1. Differences in sweat loss, BM, and percentage BM loss between the dehydrated (DEH) and hydrated (HYD) trial at 0, 60, and 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$.

Time (min)	HYD			DEH		
	0	60	120	0	60	120
Sweat loss (L)		0.8 $\pm$ 0.1	0.5 $\pm$ 0.1		0.7 $\pm$ 0.1	0.6 $\pm$ 0.1
BM (kg)	58.9 $\pm$ 2.9	59.0 $\pm$ 2.9	58.8 $\pm$ 2.9	58.8 $\pm$ 2.8	58.3 $\pm$ 2.8*	57.6 $\pm$ 2.7*
Percentage BM loss		—	—		0.9% $\pm$ 0.1%	2.0% $\pm$ 0.2%

Values are means $\pm$ SE ($n = 9$).

* Significantly lower than HYD and 0 min in DEH ($P < 0.05$).

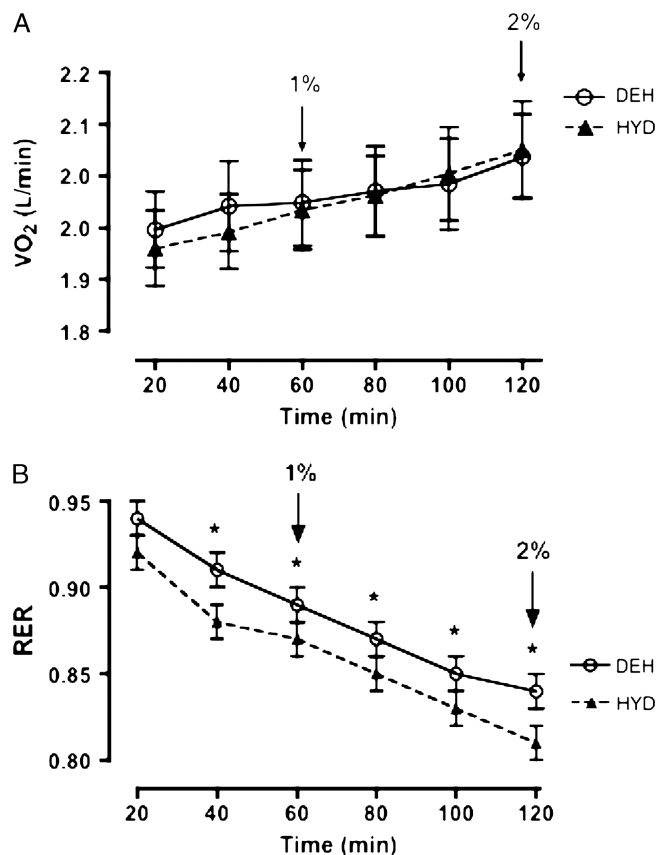


FIGURE 1—The effect of fluid intake on $\dot{V}O_2$ (A) and respiratory exchange ratio (B) during 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means $\pm$ SE ($n = 9$). $\dot{V}O_2$ and RER were significantly greater than 20 min at all time points in both trials ($P < 0.05$). *Significantly greater than HYD trial ($P < 0.05$). Arrows ($\downarrow$) indicate approximately 1% and 2% BM loss.

temperature (HYD, $22.7^\circ\text{C} \pm 0.1^\circ\text{C}$, vs. DEH, $22.5^\circ\text{C} \pm 0.1^\circ\text{C}$), relative humidity ($34\% \pm 2.9\%$ vs. $31\% \pm 3.4\%$), pretrial BM (58.9 ± 2.9 vs. 58.8 ± 2.8 kg), or hydration state (USG, 1.007 ± 0.002 vs. 1.010 ± 0.003).

BM loss, sweat loss, and fluid intake. BM was maintained in the HYD trial by drinking a mean of 1.2 ± 0.9 L of fluid over 120 min of cycling (Table 1). In the DEH trial, BM was significantly lower at 60 and 120 min of cycling, resulting in BM losses of 0.9% and 2.0% (Table 1). There were no significant differences in sweat loss between the HYD and DEH trials (Table 1). Only two of nine subjects produced urine after the HYD trial (590 and 250 mL) and only one subject after the DEH trial (160 mL).

Oxygen uptake and whole body substrate use. Mean $\dot{V}O_2$ increased in both trials with exercise time and was significantly greater than 20 min at 40, 60, 80, 100, and 120 min in both trials. There was no difference in $\dot{V}O_2$ between trials (Fig. 1A). The RER progressively decreased in both trials over time and was significantly lower than 20 min at all time points in each trial (Fig. 1B). As well, the RER was significantly higher in the DEH versus HYD trial from 40 to 120 min. CHO oxidation from 0 to 60 and 60 to 120 min

was significantly greater in the DEH (0–60 min, 111 ± 7 g; 60–120 min, 82 ± 7 g) vs. HYD trial (102 ± 7 and 73 ± 5 g), and fat oxidation was significantly lower from 0 to 60 and 60 to 120 min in the DEH (0–60 min, 17 ± 4 g; 60–120 min, 30 ± 3 g) versus HYD trial (21 ± 4 and 35 ± 3 g). Total CHO oxidation was significantly greater in the DEH (193 ± 17 g) versus HYD (175 ± 17 g) trials, and total fat oxidation was

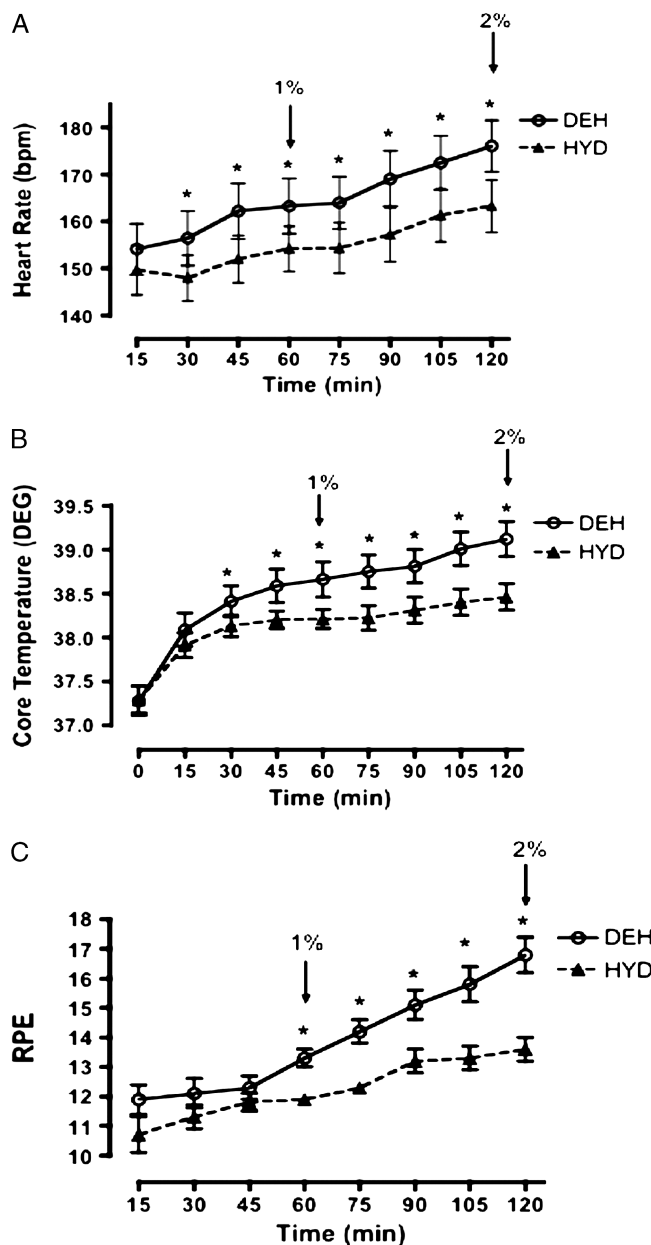


FIGURE 2—The effect of fluid intake on HR (A), Tc (B), and RPE (C) during 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means $\pm$ SE ($n = 9$). HR was significantly greater than 15 and 30 min at 45 min and beyond in both trials ($P < 0.05$). Tc was significantly greater than 15 min for all time points in both trials ($P < 0.05$). RPE was significantly greater than 15 min from 60 to 120 min in both trials ($P < 0.05$). *Significantly greater than HYD trial ($P < 0.05$). Arrows ($\downarrow$) indicate approximately 1% and 2% BM loss.

TABLE 2. Whole Hb, Hct, glucose, lactate, Pvol loss, plasma FFA, EPI, and concentration during 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials.

Time (min)	Trial	0	20	40	60	80	100	120
Hb (g·100 mL) ⁻¹	HYD	11.9 ± 0.4	12.9 ± 0.5	12.6 ± 0.3	12.8 ± 0.3	12.6 ± 0.3	12.7 ± 0.3	12.6 ± 0.3
	DEH	11.8 ± 0.4	12.9 ± 0.5	13.3 ± 0.6*	13.3 ± 0.4*	13.4 ± 0.5*	13.2 ± 0.3*	13.3 ± 0.4*
Hct (%)	HYD	40.3 ± 1	41.1 ± 0.8	41.2 ± 1	42.3 ± 1.5	42.2 ± 0.9	42.4 ± 0.8	42.0 ± 0.9
	DEH	39.8 ± 1.1	41.6 ± 1.2	41.6 ± 1.1	43.0 ± 0.8	42.8 ± 0.9	42.8 ± 0.9	44.3 ± 0.7*
Pvol loss (%)	HYD	—	-3.6 ± 0.9	-4.6 ± 0.7	-6.2 ± 1.9	-5.5 ± 1.5	-5.8 ± 1.0	-5.3 ± 1.2
	DEH	—	-3.3 ± 1.4	-8.2 ± 0.9*	-9.6 ± 1.1*	-9.5 ± 0.9*	-10.1 ± 1.1*	-11.3 ± 1.2*
Glucose (18)	HYD	4.5 ± 0.1	4.1 ± 0.2	4.0 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.2
	DEH	4.5 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Lactate (18)	HYD	0.8 ± 0.1	1.5 ± 0.4	1.3 ± 0.7	1.3 ± 0.3	1.0 ± 0.2	1.4 ± 0.4	1.0 ± 0.4
	DEH	0.8 ± 0.1	2.2 ± 0.6*	1.9 ± 0.5*	2.0 ± 0.6*	2.3 ± 0.7*	2.0 ± 0.6*	2.4 ± 0.7*
Plasma FFA (18)	HYD	0.2 ± 0.04	—	—	0.4 ± 0.1	0.6 ± 0.1	—	1.0 ± 0.2
	DEH	0.2 ± 0.04	—	—	0.3 ± 0.1	0.4 ± 0.1	—	0.7 ± 0.1
Plasma EPI (nM)	HYD	0.8 ± 0.1	—	—	1.3 ± 0.2	1.4 ± 0.2	—	1.5 ± 0.3
	DEH	0.7 ± 0.1	—	—	1.2 ± 0.1	1.3 ± 0.1	—	1.4 ± 0.2

Values are means ± SE (*n* = 9).

* Significantly greater than HYD (*P* < 0.05).

Blood Hb, Hct, Pvol loss, blood lactate, plasma FFA, and EPI were significantly greater than 0 min at all time points in both trials (*P* < 0.05). Blood glucose was significantly lower than 0 min from 40 to 120 min in both trials (*P* < 0.05).

significantly lower in the DEH (47 ± 1 g) versus HYD (56 ± 1 g) trials.

Cardiovascular, thermoregulatory, and RPE responses. HR significantly increased over time in both trials and became significantly greater than 15 and 30 min at 45 min and beyond in both trials. Subjects had a significantly higher HR from 30 to 120 min of cycling in the DEH versus HYD trial (Fig. 2A). Tc was significantly greater than 15 min for all time points in both trials. In the DEH trial, Tc was significantly greater than the HYD trial from 30 to 120 min (Fig. 2B). RPE significantly increased over time in both trials and was significantly greater than 15 min from 60 to 120 min. RPE was significantly greater in the DEH versus HYD trial from 60 to 120 min (Fig. 2C). The mean RPE over the entire 120-min trial was significantly greater in the DEH trial (DEH, 13.9 ± 0.6, vs. HYD, 12.3 ± 0.4).

Blood measurements. Blood Hb and Hct were significantly higher than rest from 20 to 120 min of exercise in both trials (Table 2). In the DEH trial, Hb was significantly greater than the HYD trial from 40 to 120 min (Table 2). There was a trend for Hct to be higher in the DEH trial throughout the exercise, but the difference did not reach significance until 120 min of exercise (Table 2). Pvol loss was significantly greater than rest in both trials at all exercise time points and significantly greater in the DEH versus

HYD trial from 40 to 120 min (Table 2). Blood glucose was significantly lower than rest between 40 and 120 min in both trials, with no difference between trials (Table 2). Blood lactate was significantly increased from rest at 20 min of exercise and beyond in both trials and was also significantly higher in the DEH versus HYD trial at all exercise time points (Table 2). Plasma FFA and EPI significantly increased from rest at all time points in both trials, with no significant differences between trials (Table 2).

Muscle fuels and metabolites. Skeletal muscle PCr significantly decreased in the first 60 min of exercise in both trials and remained significantly lower than rest at 120 min of exercise in both trials, but was not significantly different between trials (Table 3). Cr increases were reciprocal with the PCr decreases, and muscle ATP content was not significantly changed with exercise or between trials (Table 3). Muscle free ADP and AMP significantly increased in the first 60 min of exercise in both trials and remained significantly higher than rest at 120 min of exercise in both trials, but was not significantly different between trials (Table 3). Muscle lactate content increased with exercise and peaked at 60 min in both trials and was significantly greater at 60 min in the DEH versus HYD trial (Table 3). Muscle glycogen content was similar in the two trials before exercise and significantly lower at 60 and 120 min in both

TABLE 3. Skeletal muscle fuel and metabolite contents during 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials.

Time (min)	HYD			DEH		
	0	60	120	0	60	120
PCr (mmol·kg ⁻¹ dm)	80.1 ± 4.3	60.1 ± 6.2	55.7 ± 7.4	81.1 ± 4.1	59.3 ± 5.6	56.0 ± 6.9
Cr (mmol·kg ⁻¹ dm)	81.0 ± 3.8	101.0 ± 5.2	105.3 ± 8.9	80.2 ± 3.8	102.0 ± 7.8	105.5 ± 6.7
ATP (mmol·kg ⁻¹ dm)	24.9 ± 1.2	23.3 ± 1.0	24.4 ± 1.3	23.9 ± 1.4	24.1 ± 3.5	24.9 ± 1.7
ADP (μmol·kg ⁻¹ dm)	133.1 ± 11.8	230.5 ± 27.7	246.7 ± 49.4	134.0 ± 5.5	231.0 ± 32.1	279.9 ± 43.1
AMP (μmol·kg ⁻¹ dm)	0.7 ± 0.1	2.5 ± 0.7	3.4 ± 1.3	0.7 ± 0.1	2.3 ± 0.5	3.5 ± 0.9
Lactate (mmol·kg ⁻¹ dm)	3.3 ± 0.3	6.1 ± 1.1	7.1 ± 1.2	3.3 ± 0.6	10.2 ± 2.3*	8.4 ± 1.7
Glycogen (mmol·kg ⁻¹ dm)	425 ± 36	223 ± 41	173 ± 34	461 ± 28	223 ± 26	131 ± 18

Values are means ± SE (*n* = 9).

* Significantly higher than HYD trial (*P* < 0.05).

PCr and glycogen were significantly lower than 0 at 60 and 120 min. Cr, ADP, AMP, and lactate were significantly greater than 0 at 60 and 120 min.

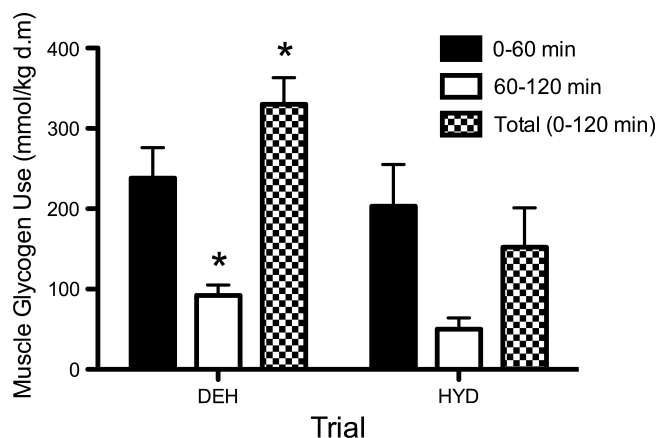


FIGURE 3—Muscle glycogen use during 120 min of cycling at approximately 65% $\dot{V}O_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means $\pm$ SE ($n = 9$). *Significantly greater than HYD trial ($P < 0.05$).

trials compared with rest (Table 3). There was no significant difference in glycogen use (17%) in the first 60 min of exercise between trials (DEH, 238 ± 38 , vs. HYD, 203 ± 52 mmol·kg⁻¹ dry muscle (dm)). There was significantly more glycogen used (84%) from 60 to 120 min of exercise in the DEH (92 ± 13 mmol·kg⁻¹ dm) versus HYD (50 ± 14 mmol·kg⁻¹ dm) trials and significantly more glycogen used (31%) during the entire DEH (330 ± 33 mmol·kg⁻¹ dm) versus HYD (252 ± 49 mmol·kg⁻¹ dm) trials (Fig. 3).

DISCUSSION

This study investigated the effects of mild progressive dehydration during exercise at approximately 65% $\dot{V}O_{2peak}$ on whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated females. In the control trial (HYD) of this study, we maintained hydration by having subjects drink enough fluid to precisely replace their sweat losses over the 120-min cycling trial. During this trial, HR increased from 150 ± 5 bpm at 20 min to 158 ± 5 and 165 ± 5 bpm at 60 and 120 min, whereas Tc increased from $37.3^\circ\text{C} \pm 0.2^\circ\text{C}$ at rest to $37.9^\circ\text{C} \pm 0.1^\circ\text{C}$ at 15 min and $38.2^\circ\text{C} \pm 0.1^\circ\text{C}$ and $38.5^\circ\text{C} \pm 0.2^\circ\text{C}$ at 60 and 120 min. In the DEH trial, when the subjects progressively dehydrated by sweating, they lost approximately 1% and 2% BM at 60 and 120 min and added progressive dehydration to the physiological demands of exercising for 120 min at approximately 65% $\dot{V}O_{2max}$. All physiological responses to exercise were exacerbated in the DEH trial as HR increased from 154 ± 5 at 20 min to 163 ± 5 , and 176 ± 5 bpm at 60 and 120 min, representing increases of 4–9 bpm at these time points. Subjects also had elevated Tc values ($37.3^\circ\text{C} \pm 0.2^\circ\text{C}$ at rest and $38.1^\circ\text{C} \pm 0.2^\circ\text{C}$, $38.7^\circ\text{C} \pm 0.2^\circ\text{C}$, and $39.1^\circ\text{C} \pm 0.2^\circ\text{C}$ at 20, 60, and 120 min) and were 0.2°C, 0.5°C, and 0.6°C higher at 20, 60 and 120 min in the DEH versus HYD

trial. Even in the first hour of exercise in DEH (approximately 1% BM loss), RPE, Pvol loss, and blood (La) were all higher, and there was a significantly greater reliance on whole body carbohydrate, higher muscle lactate content, and a trend for higher muscle glycogen use ($P = 0.15$). In the second hour, BM loss progressed from 1% to 2%, and the additional physiological parameters remained higher, and whole body carbohydrate oxidation and muscle glycogen use were also significantly greater in the DEH trial. The 2% BM loss for 2 h of exercise increased whole body carbohydrate oxidation by 9% and muscle glycogen use by 31% in female subjects who were hydrated before exercise.

The effects of dehydration on substrate oxidation and muscle metabolism. Hargreaves et al. (17) demonstrated in trained males that a 3% BM loss over a 2-h trial resulted in a significantly higher whole body RER after 60 and 120 min of exercise compared with the euhydrated trial. They also observed a 16% greater muscle glycogen use over the entire trial with fluid restriction. Similar results were reported by Gonzalez-Alonso et al. (15) who had male subjects cycle until volitional exhaustion (135 ± 4 min) in the heat (35°C) while progressively dehydrating to approximately 3.9% BM loss. They reported increased carbohydrate oxidation, muscle glycogen use (45% greater), muscle lactate accumulation, and net lactate release across the contracting leg compared with the euhydrated trial. In contrast, Walsh et al. (37) demonstrated that mild dehydration of 1.3% BM loss in trained males did not change RER after 60 min of cycling at 70% $\dot{V}O_{2peak}$ in the heat (32°C). It seems likely that the low level of dehydration may be the reason for no effect on RER. In comparison, the present study with a neutral environment, which was conducted on recreational trained females, demonstrated that RER was significantly higher in the DEH trial as early as 40 min of cycling when dehydration was <1% BM loss and remained significantly higher than the HYD trial for the duration of the trial. The present RER and glycogenolysis data matches Hargreaves et al. (17) despite this study being conducted on recreationally trained females and not trained males. As well, our results suggest that a portion of the increased pyruvate production in the DEH trial was oxidized and some was converted to lactate.

The major question is, “What accounts for the increased glycogenolysis in the DEH trial?” There are three main hypotheses that have been proposed to explain the substrate shift toward greater carbohydrate metabolism and muscle glycogenolysis during exercise and heat stress; 1) an augmented sympathoadrenal response leading to greater glycogen phosphorylase (PHOS) activation and flux, 2) increased allosteric activation of glycogen PHOS via increased free ADP and AMP (energy status of the cell) levels, and 3) higher intramuscular temperature during exercise when dehydrated (8). Hargreaves et al. (17) reported no difference in plasma EPI at 60 or 120 min of cycling with 3% BM loss but significantly greater plasma norepinephrine content only at 120 min. The authors suggested that fluid ingestion during exercise

attenuates the normal exercise-induced increase in EPI, and the blunting of the sympathoadrenal activity may be due to hydration status and Tc, but it is difficult to assess these factors independently. The present study also found no difference in EPI response with fluid restriction (2% BM loss) in females, which downplays the role of EPI as the reason for the significantly greater muscle glycogen use and muscle lactate content at 60 min while exercising dehydrated.

The energy status of the cell (free ADP and AMP) exerts powerful allosteric regulation of glycogen PHOS and therefore plays a vital role in determining the rate of glycogenolysis. In light of the augmented glycogen use accompanying progressive DEH, we predicted that the energy status of the cell may be decreased (higher free ADP and AMP) to a greater extent when dehydrated and explain the accelerated glycogen use. However, this was not observed, suggesting that the energy status of the cell was not altered by mild dehydration.

Previous work suggests that higher Tc and Tm are responsible for the increased glycogenolysis and increased reliance on carbohydrate oxidation for muscle ATP production (9–11,15,34). Research investigating local hyperthermia in the working muscle demonstrated an increase in Tm, muscle glycogenolysis, and muscle (La), independent of changes in circulating EPI (17,32). Starkie et al. (34) cooled one leg before two-legged cycling and reported increased glycogenolysis in noncooled or hotter leg when both legs were exposed to the same (EPI). Febbraio (9) concluded in a review that increases in Tc of $>0.5^{\circ}\text{C}$ significantly increased intramuscular carbohydrate use during moderate intensity exercise in the heat. In the present study, in a neutral environment, Tc was already 0.2°C – 0.5°C higher from 20 to 60 min of exercise in the DEH trial. Although Tm was not measured in this study, the work from Hargreaves' laboratory (7,9,34) suggests that Tm would also have been higher in the first hour of exercise of the present study. Therefore, Tm (Q10 effect) appears to be the primary mechanism inducing the shift in intramuscular glycogenolysis and whole body carbohydrate oxidation and during progressive dehydration in females. It is currently unknown why dehydration preferentially increases CHO metabolism and not fat metabolism and future research needs to further elucidate the effect of dehydration on the perturbations to intramuscular metabolism. One would predict that the Q10 effect would also increase fat metabolism; however, the results of this study demonstrate that fat oxidation was reduced with mild dehydration, and carbohydrate oxidation was more sensitive than fat oxidation to increases in Tc. The down-regulation of fat oxidation cannot be explained by FFA delivery because there was no difference between trials in plasma FFA, nor can the effect of muscle pH on carnitine palmitoyl transferase 1 be considered as a mechanism for the decreased fat oxidation because there was no significant difference in muscle acidity between trials. This has been reported in the past because Montain et al. (23) had subjects perform knee-kicking exercise to exhaustion (approximately 250 s) when hypohydrated to 4% BM or euhydrated. Al-

though performance was reduced by 15% in the hypohydrated trial (213 vs. 251 s), they reported no change in muscle pH or ATP levels between trials. Moreover, mild dehydration may affect the uptake of FFA into the muscle or alter intramuscular triacylglycerol breakdown; however, this is merely speculation, and the mechanisms by which dehydration causes a reduction in fat oxidation remain unclear and call for further investigation.

Effects of dehydration on cardiovascular and thermal responses. It is well established that fluid ingestion attenuates the increases in HR and Tc and the decreases in stroke volume and cardiac output that occur during prolonged exercise without fluid ingestion (1,2,6,11,16,19,20,32). An early study demonstrated that when heat-acclimatized male subjects were dehydrated to 3%, 5%, and 7% BM loss by an exercise-heat regime and then walked in a hot environment (49°C) at a low intensity for 140 min, HR and Tc increased linearly with the severity of dehydration (32). In a similar way, our results demonstrated that as dehydration increased from 0% to 1% and 1% to 2% BM during exercise in the DEH trial, HR and Tc became progressively higher than the elevations in the HYD trial. Hypovolemia and the displacement of blood to the skin for evaporative cooling make it difficult to maintain central venous pressure (CVP) during exercise when fluid is restricted (31). CVP is regulated by the continuous adjustment of blood volume to the changing size of the vascular bed to maintain cardiac output, and heat stress and/or exercise-induced dehydration provides a threat to this control because inadequate fluid intake during periods of sweat loss reduces Pvol (24). In light of the significantly greater loss in Pvol found in the DEH versus the HYD trial after approximately 20 min of cycling, a reduction in CVP and stroke volume may account for the significantly elevated HR to maintain cardiac output when stroke volume was compromised. An accompanying baroreflex that would decrease cutaneous blood flow and heat transfer to the periphery leading to heat storage may account for the augmented Tc found in the DEH trial. In support of this, Nadel et al. (25) reported that diuretic-induced dehydration of 2.7% BM loss led to restrictions in core-to-skin heat transfer, which forced esophageal temperature to nearly 39°C during 30 min of cycling at $55\% \dot{V}\text{O}_{2\text{peak}}$ in the heat compared with 38.4°C in euhydrated subjects. Montain and Coyle (22) investigated whether fluid ingestion attenuated the hyperthermia and cardiovascular drift that occurred during exercise dehydration due to increases in blood volume. Seven trained male subjects exercised at approximately $65\% \dot{V}\text{O}_{2\text{peak}}$ for 2 h in three conditions; no fluid replacement, infusion with a blood volume expander, or given fluid to replace approximately 80% of sweat loss. They reported that fluid replacement and the blood volume expander treatment maintained blood volume compared with the no fluid trial, but only fluid replacement resulted in lower Tc. The authors argued that the decreased hyperthermia during exercise in the fluid replacement trial was due to the measured increase in skin blood flow. In the present study, female subjects had higher Tc values in the last 90 min of exercise in the

DEH versus HYD trials, whereas the sweat rates were similar, suggesting that the lack of heat transfer to the periphery accounted for the elevated Tc in the DEH trial with as little as 1%–2% BM loss.

Effects of dehydration on ratings of perceived exertion. In this study, RPE mirrored the rise in HR and Tc with progressive dehydration and became significantly higher in the DEH trial at 60 min of cycling when subjects had lost approximately 1% BM. Similar results have been reported in other studies investigating the effects of progressive dehydration on RPE (16,20). It is speculated that hypovolemia associated with exercise dehydration leading to a reduction in brain blood flow may exasperate the displeasure associated with exercising without fluid leading to greater perceived exertion (18). More simply, it may be that the elevations in Tc, HR, and reduced Pvol in the DEH trial are sensed, and the feedback to the brain results in the greater RPE during exercise at the same relative intensity in a mildly dehydrated state. Shirreffs et al. (33) reported that as subjects became progressively more dehydrated to 2.7% BM loss, they reported feelings of headache and reductions in their ability to concentrate and their alertness was reduced, which are all contributing factors to an elevated RPE during exercise.

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CONCLUSIONS

This study is the first to investigate the time course of changes in whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated females with progressive mild dehydration during exercise at approximately 65% $\dot{V}O_{2peak}$ in a neutral environment. Moreover, total carbohydrate oxidation and muscle glycogenolysis were significantly increased early in exercise when BM loss was <1% to 2%, which we attribute to dehydration-induced increases in Tc and skeletal Tm, because there were no differences in plasma EPI or the energy status of the cell (free ADP or AMP) between the HYD and DEH trials. In addition, the traditional changes in physiological parameters accompanying exercise in a HYD state were exacerbated with mild dehydration of 1%–2% BM loss.

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